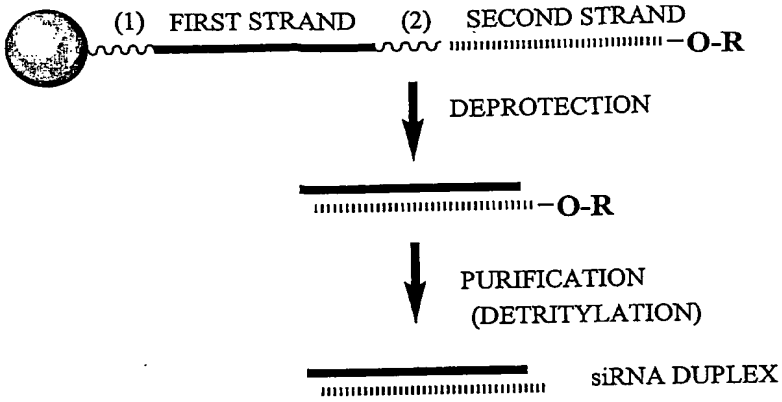
Figure 1

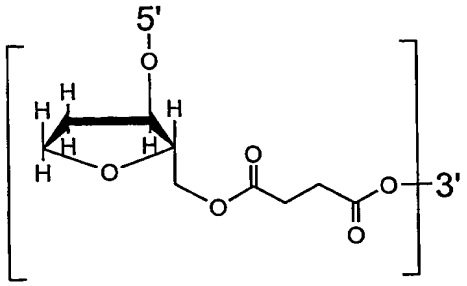


= SOLID SUPPORT

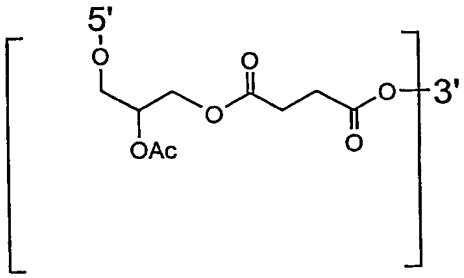
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

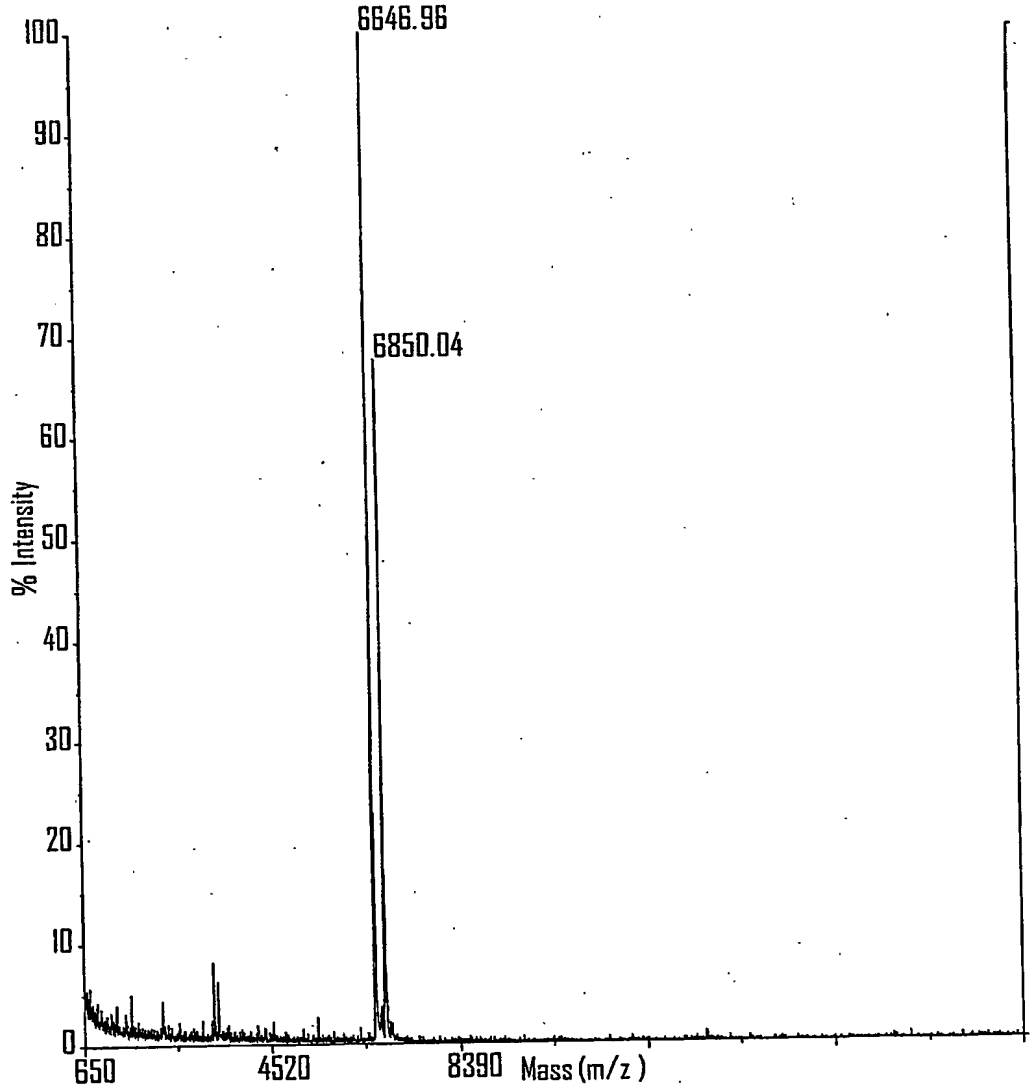

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

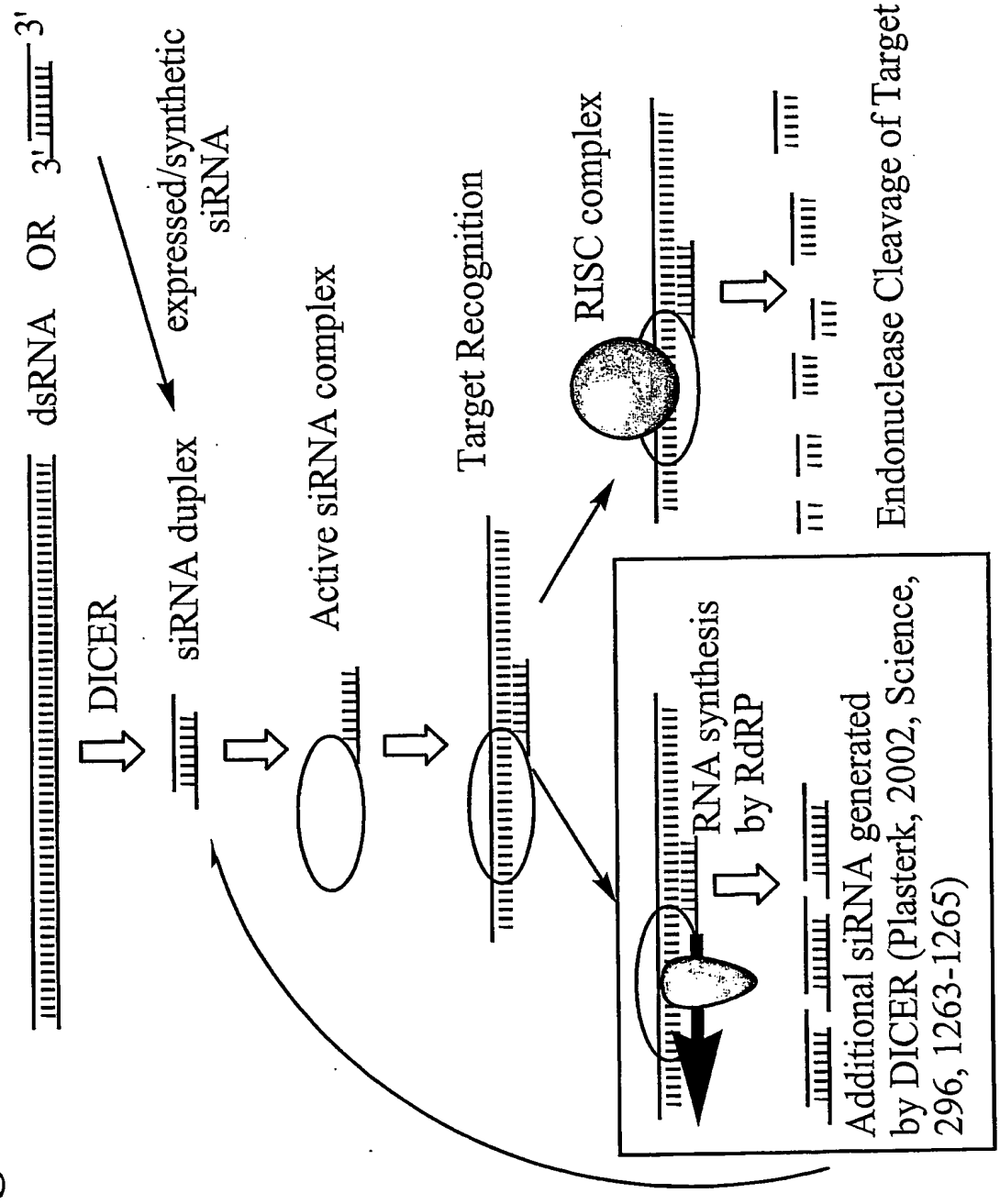

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Endonuclease Cleavage of Target
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)

Figure 4

A

SENSE STRAND (SEQ ID NO 1883)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 1884)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 1885)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-OM EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N_SN) -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 1886)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 1887)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 1888)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 1889)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 1886)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 1890)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N N) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 1886)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 1889)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 1891)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL OR B THAT IS OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE THAT IS OPTIONALLY ABSENT

Figure 5

A

SENSE STRAND (SEQ ID NO 1892)

5'- iB-C A U G G C U G C C A U C U G C G C C *T T*-iB -3'

3'- L-T_S*T* G U A C C G A C G G U A G A C G C G G -5'

ANTISENSE STRAND (SEQ ID NO 1893)

B

SENSE STRAND (SEQ ID NO 1894)

5'- *c a u g g c u g c c a u c u g c g c c* T_S*T* -3'

3'- L-T_S*T g u a c c g a c g g u a g a c g c g g* -5'

ANTISENSE STRAND (SEQ ID NO 1895)

C

SENSE STRAND (SEQ ID NO 1896)

5'- iB-c A u G G c u G c c A u c u G c G c c *T T*-iB -3'

3'- L-T_S*T G u A c c G A c G G u A G A c G c G G* -5'

ANTISENSE STRAND (SEQ ID NO 1897)

D

SENSE STRAND (SEQ ID NO 1898)

5'- iB-*c A u G G c u G c c A u c u G c G c c T T*-iB -3'

3'- L-T_S*T g u a c c g a c g g u a g a c g c g g* -5'

ANTISENSE STRAND (SEQ ID NO 1895)

E

SENSE STRAND (SEQ ID NO 1899)

5'- iB-*c* A *u* G G *c u* G *c c* A *u c u* G *c* G *c c T T*-iB -3'

3'- L-T_S*T g u a c c g a c g g u a g a c g c g g* -5'

ANTISENSE STRAND (SEQ ID NO 1895)

F

SENSE STRAND (SEQ ID NO 1898)

5'- iB-*c A u G G c u G c c A u c u G c G c c T T*-iB -3'

3'- L-T_S*T G u A c c G A c G G u A G A c G c G G* -5'

ANTISENSE STRAND (SEQ ID NO 1900)

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

italic lower case = 2'-deoxy-2'-fluoro

underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY

iB = INVERTED DEOXYABASIC

L = GLYCERYL MOIETY OR iB OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE OPTIONALLY PRESENT

Figure 6

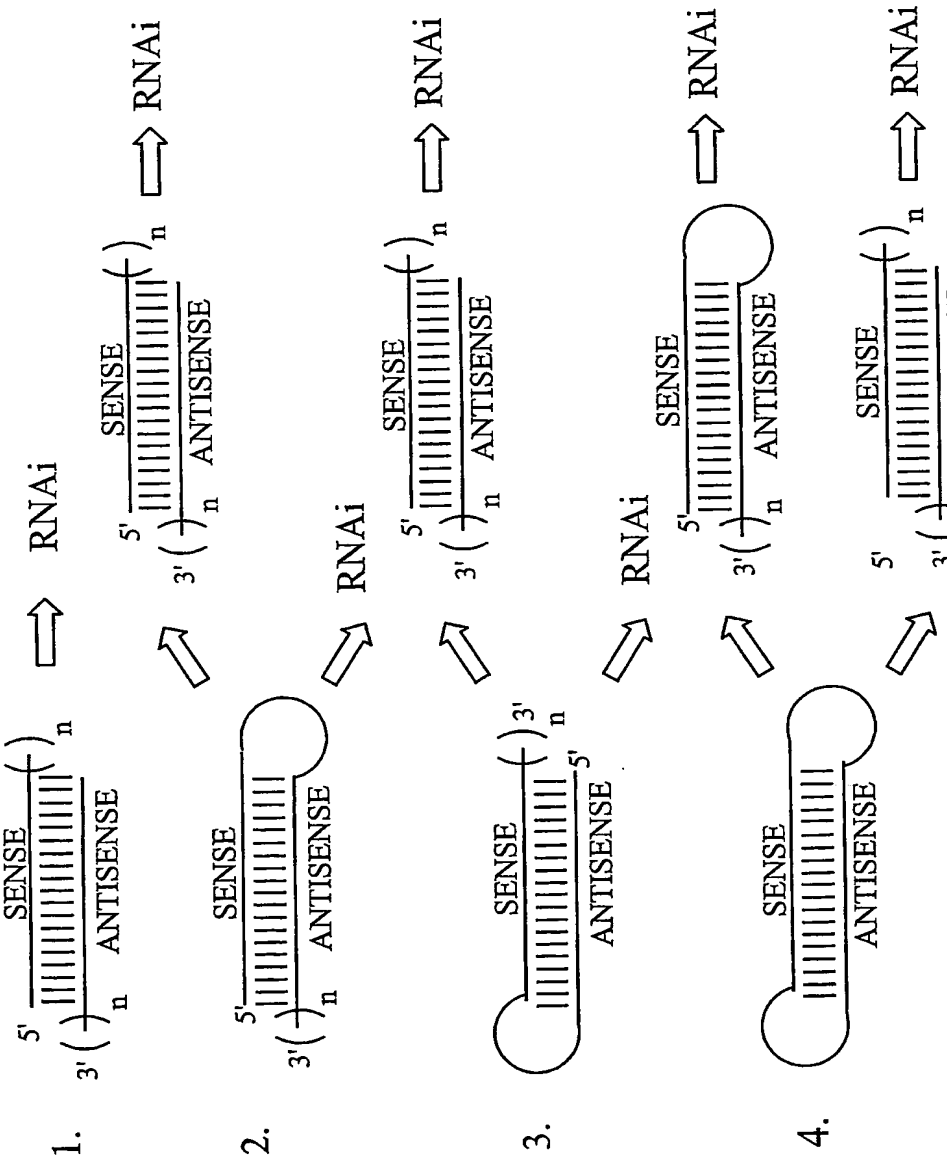


Figure 7

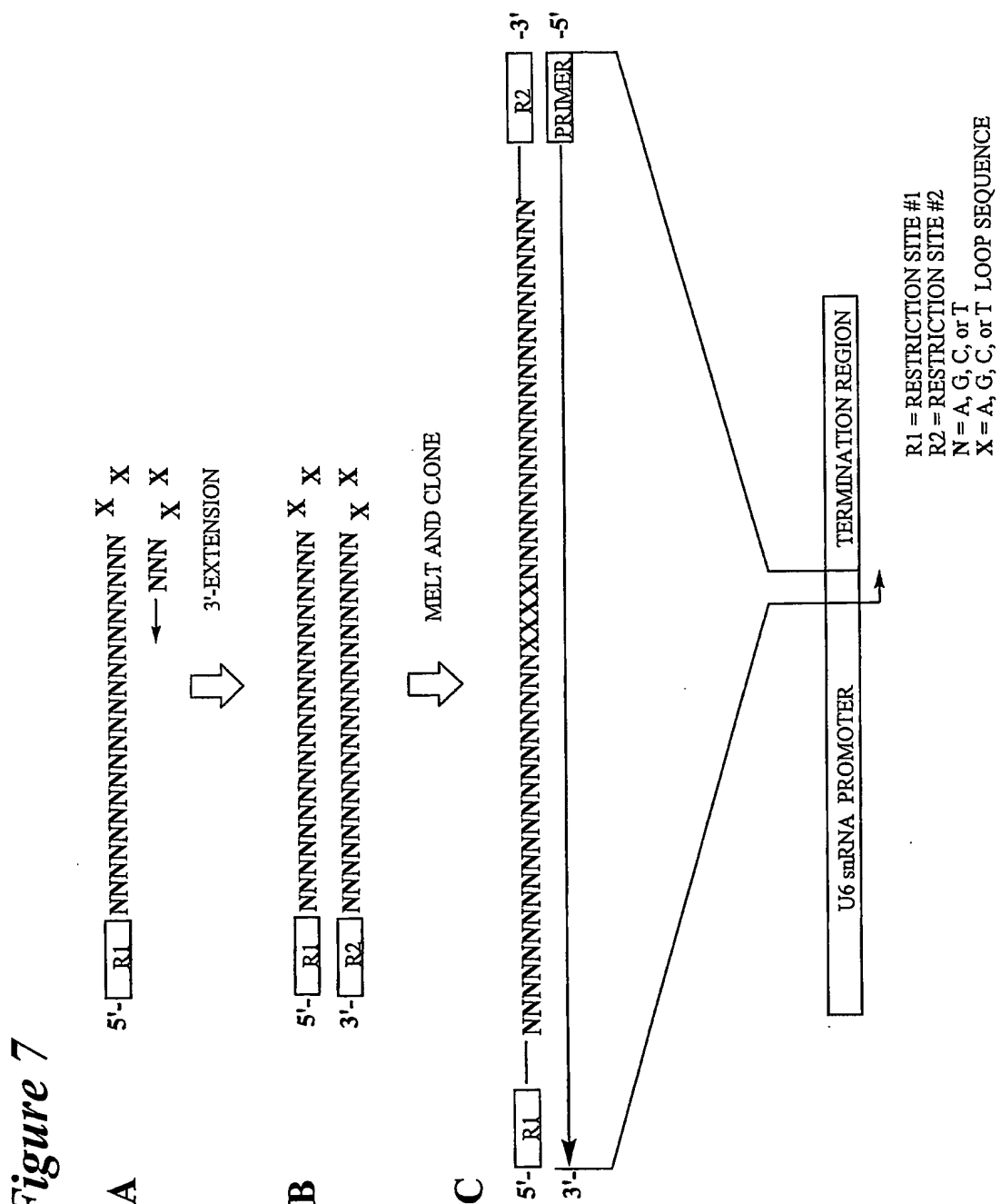
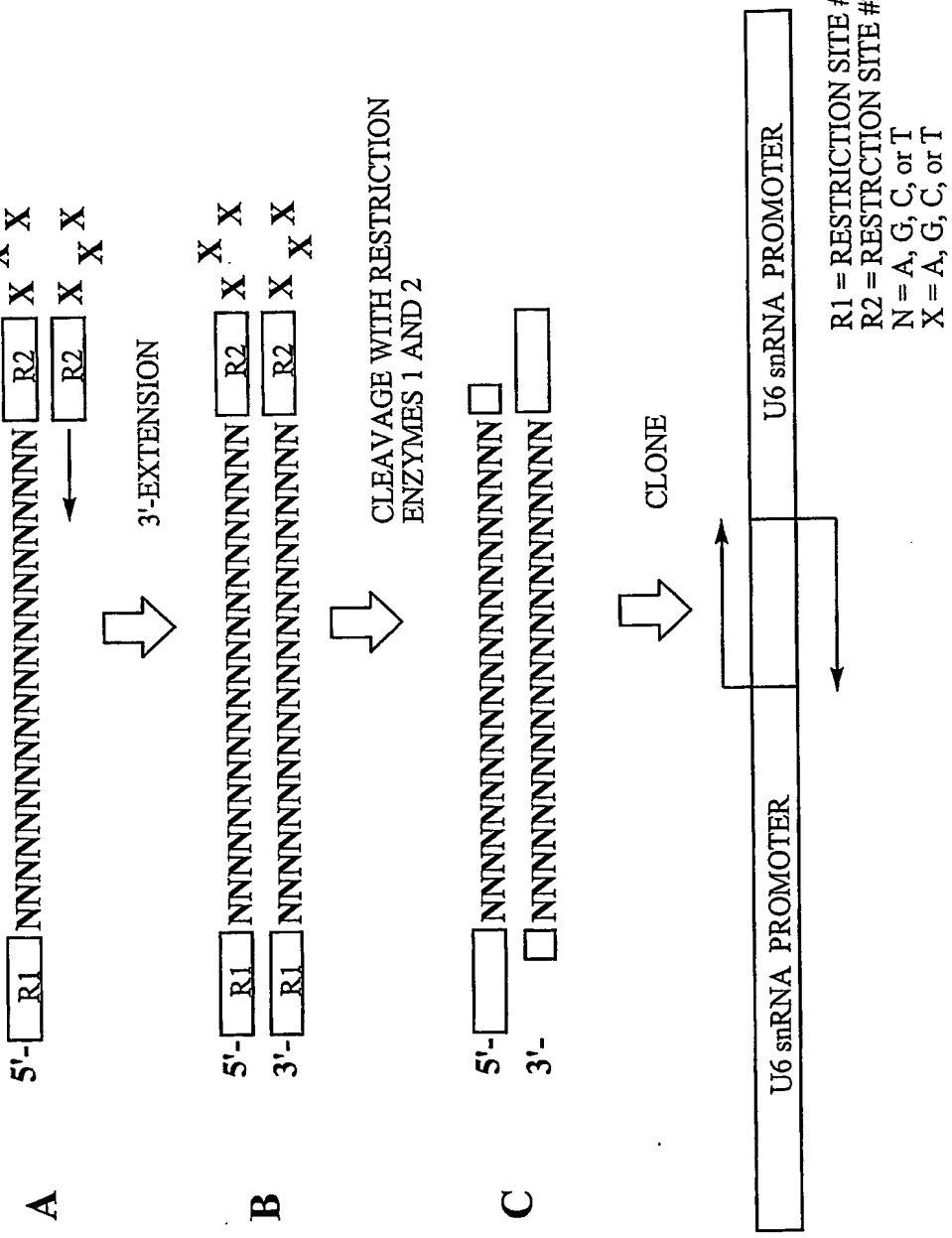

A
5'- R1 NNNNNNNNNNNNNNNNNNNN X X
NNN X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNNN X X
3'- R2 NNNNNNNNNNNNNNNNNNNN X X
MELT AND CLONE
C
5'- R1 NNNNNNNNNNNNNNNNNNNNXXXXNNNNNNNNNNNNNNNNNNNN R2 -3'
3'- PRIMER -5'
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8


A
5'-
R1
NNNNNNNNNNNNNNNNNNNN
R2
X X
X X
3'-EXTENSION
B
5'-
3'-
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
5'-
3'-
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRCTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

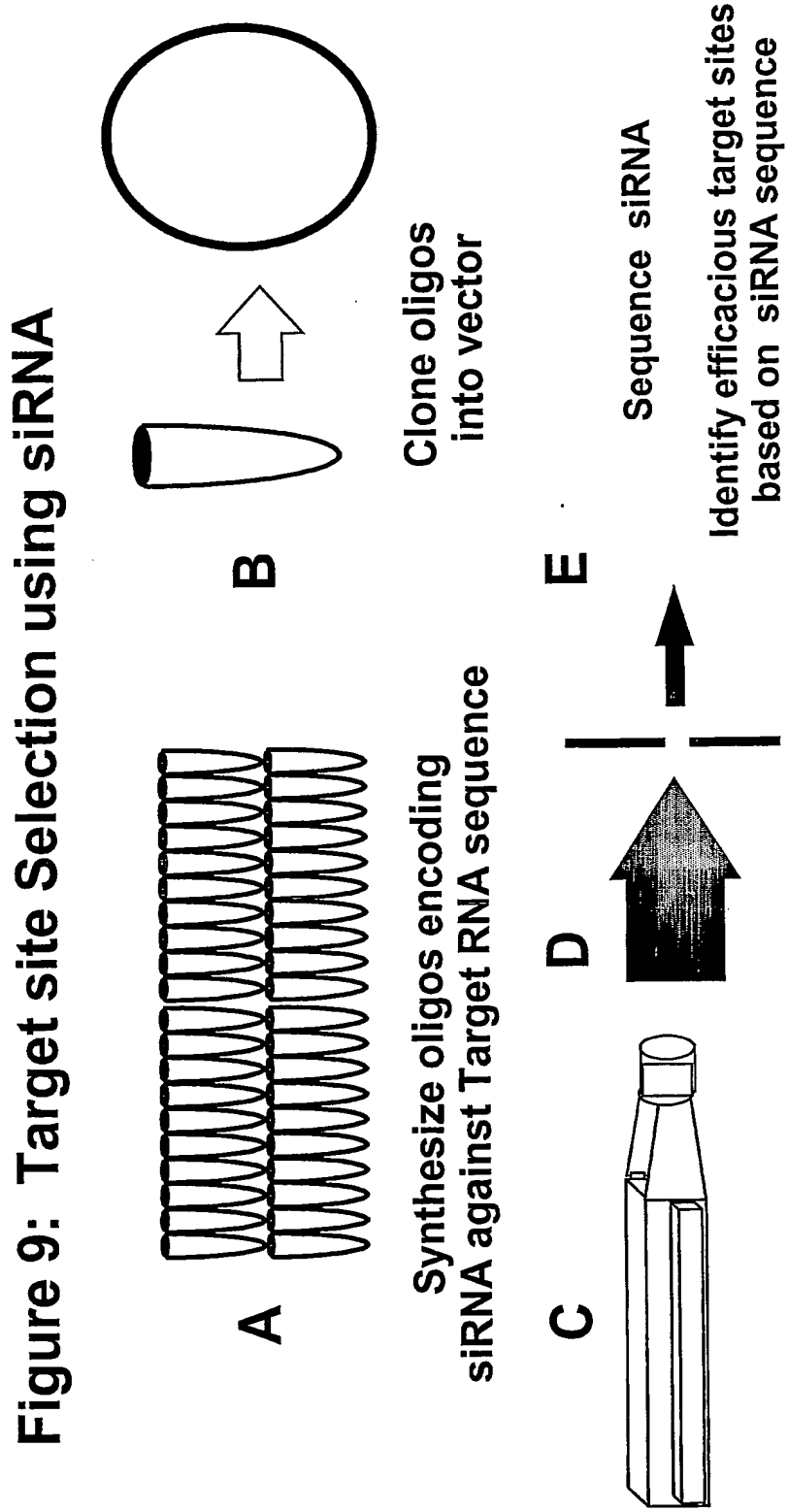
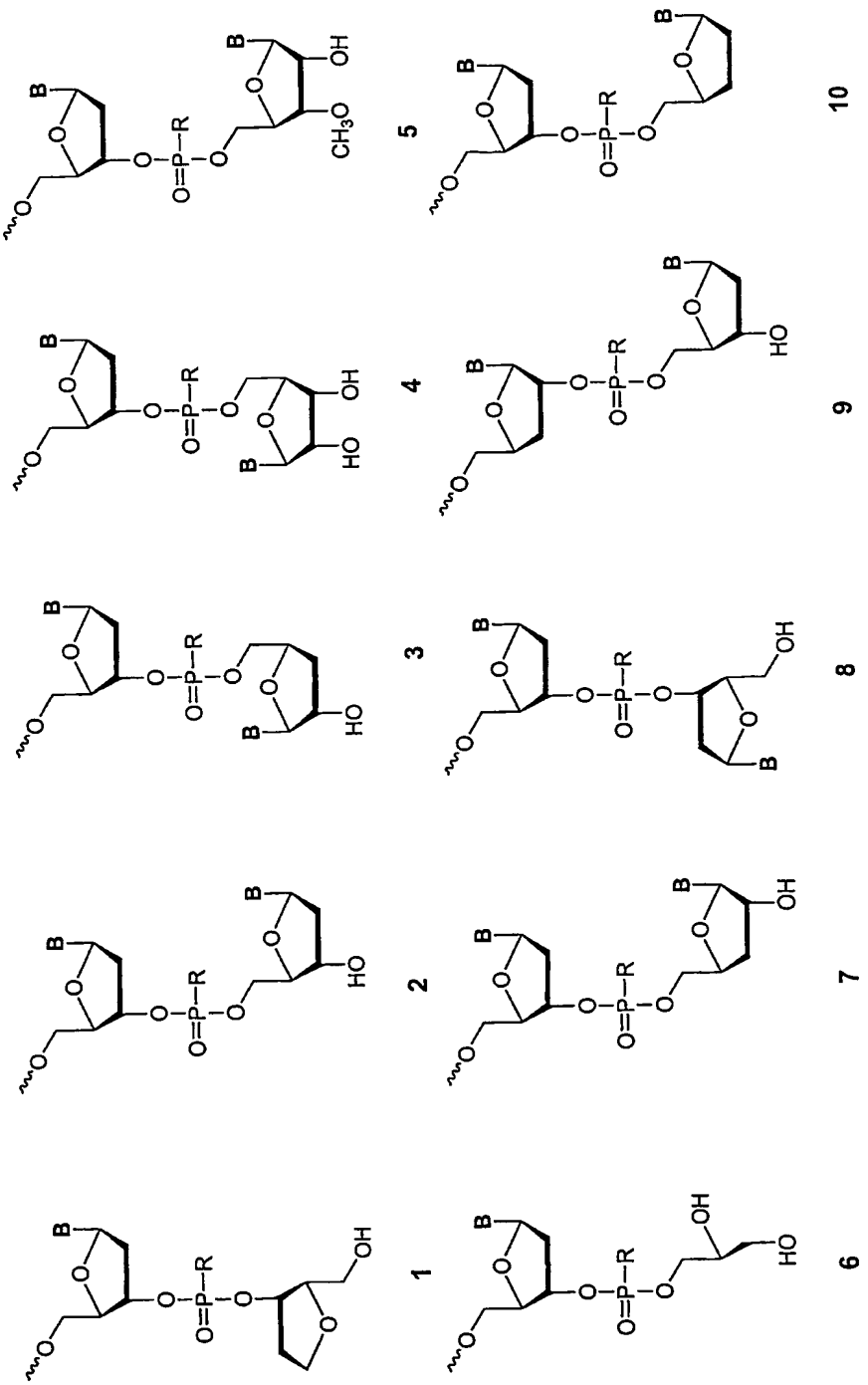


Figure 9: Target site Selection using siRNA
A
Synthesize oligos encoding siRNA against Target RNA sequence
B
Clone oligos into vector
C
Transduce target cells
D
Select cells exhibiting desired phenotype
E
Sequence siRNA
Identify efficacious target sites based on siRNA sequence

Figure 10



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

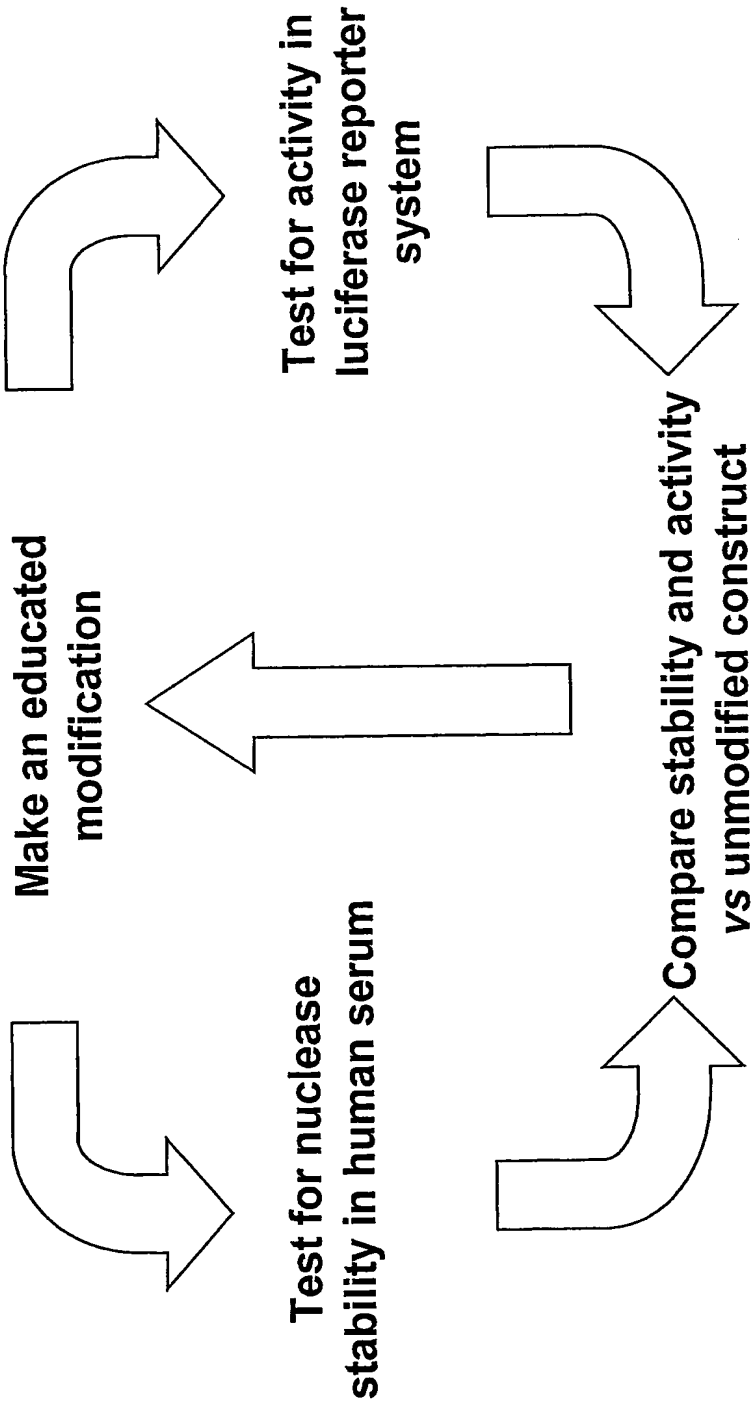
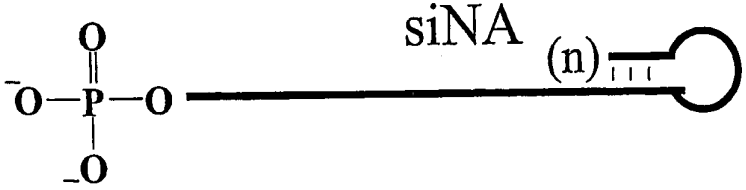


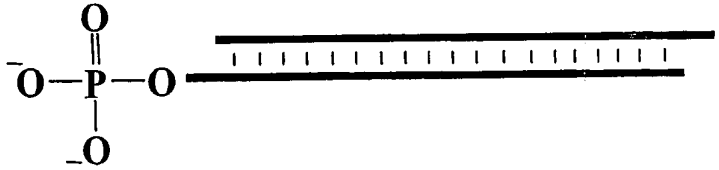
Figure 12: Phosphorylated siNA constructs

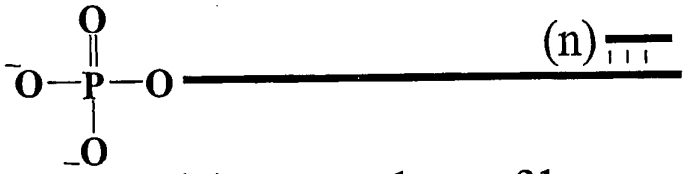
Phosphates can be modified as described herein

Asymmetric hairpin siNA



Asymmetric duplex siNA





(n) = number of base pairs (e.g. 3-18 bp)

Figure 13: 5'-phosphate modifications

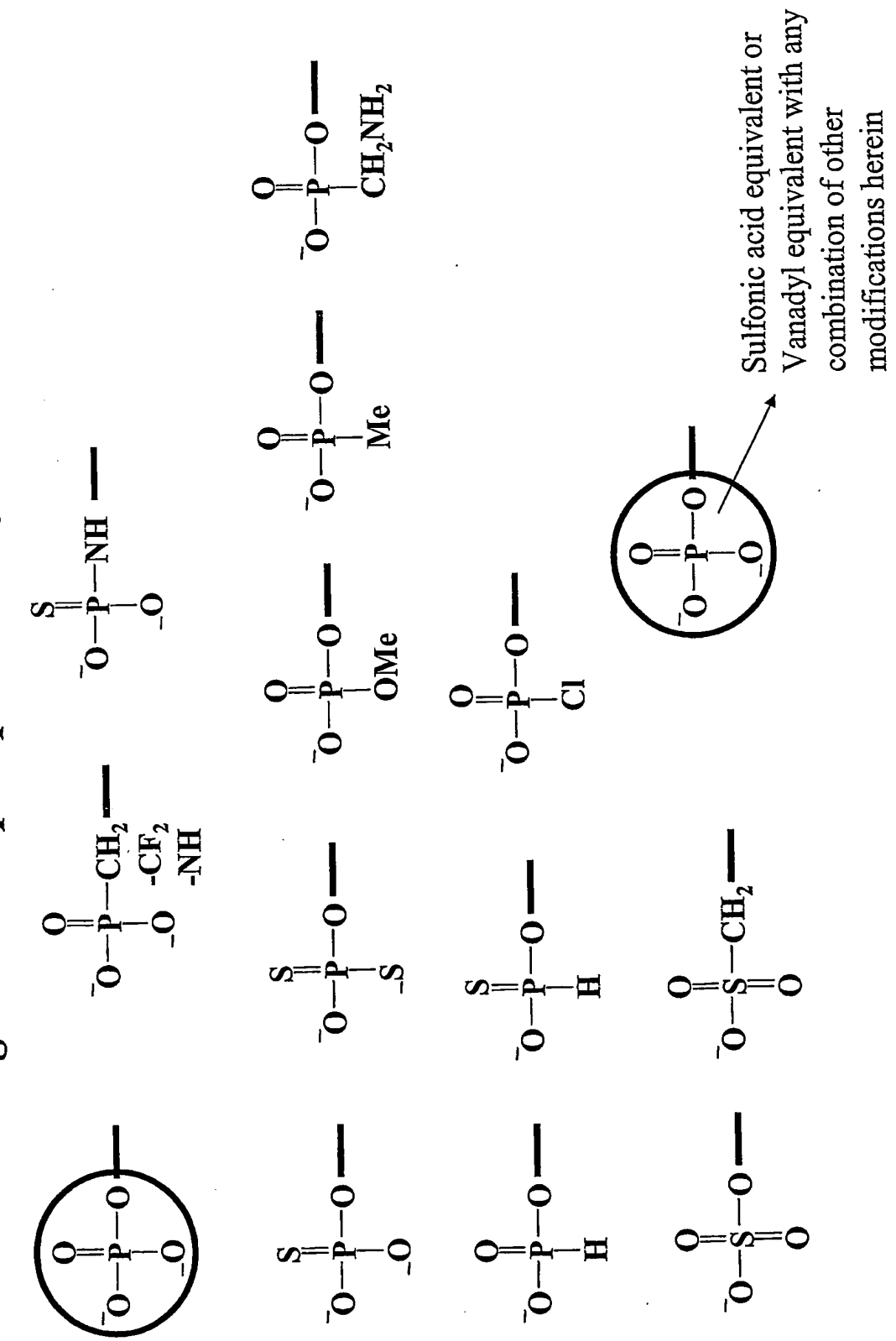


Figure 14A: Duplex forming oligonucleotide constructs that utilize Palindrome or repeat sequences

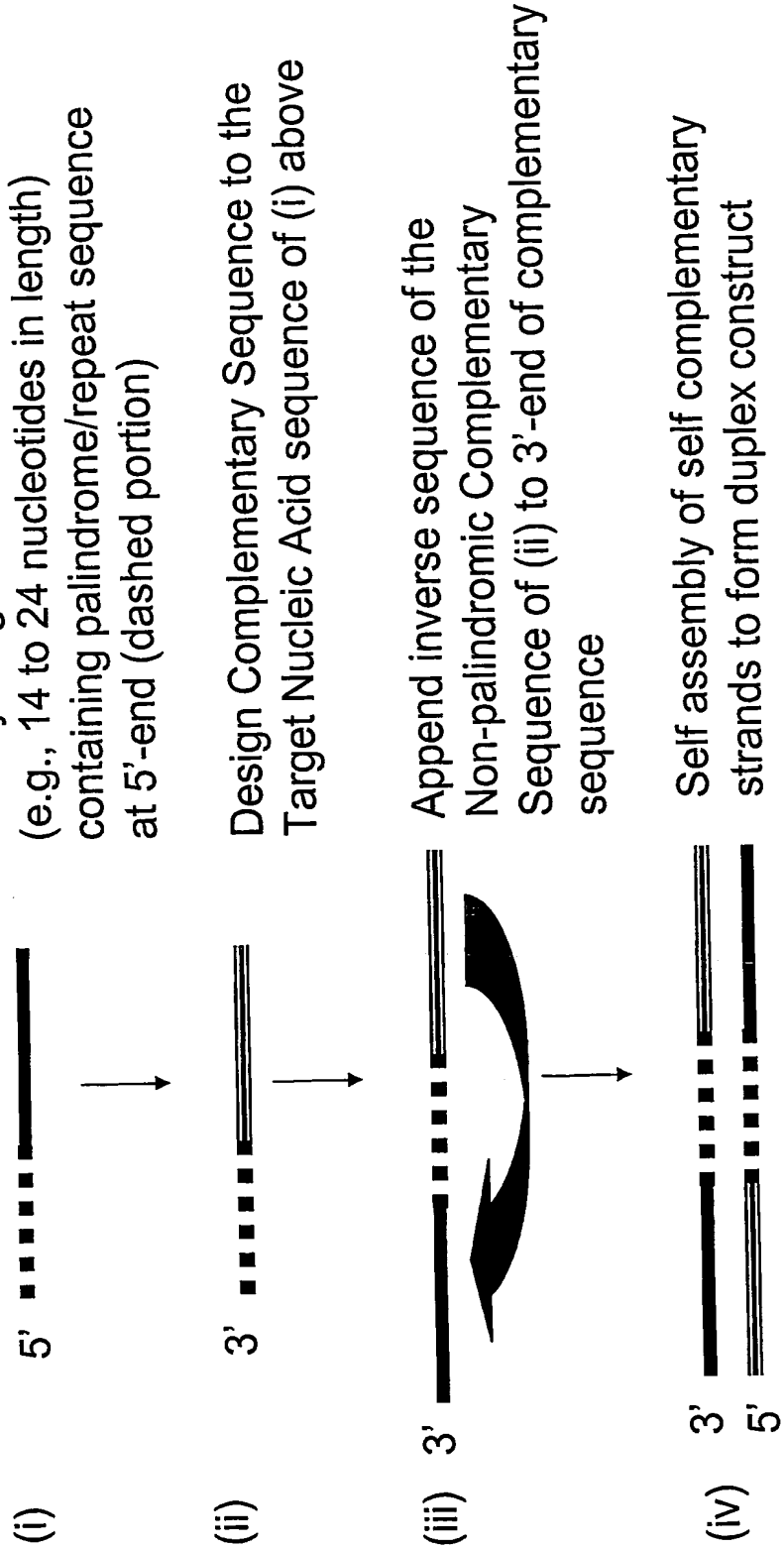
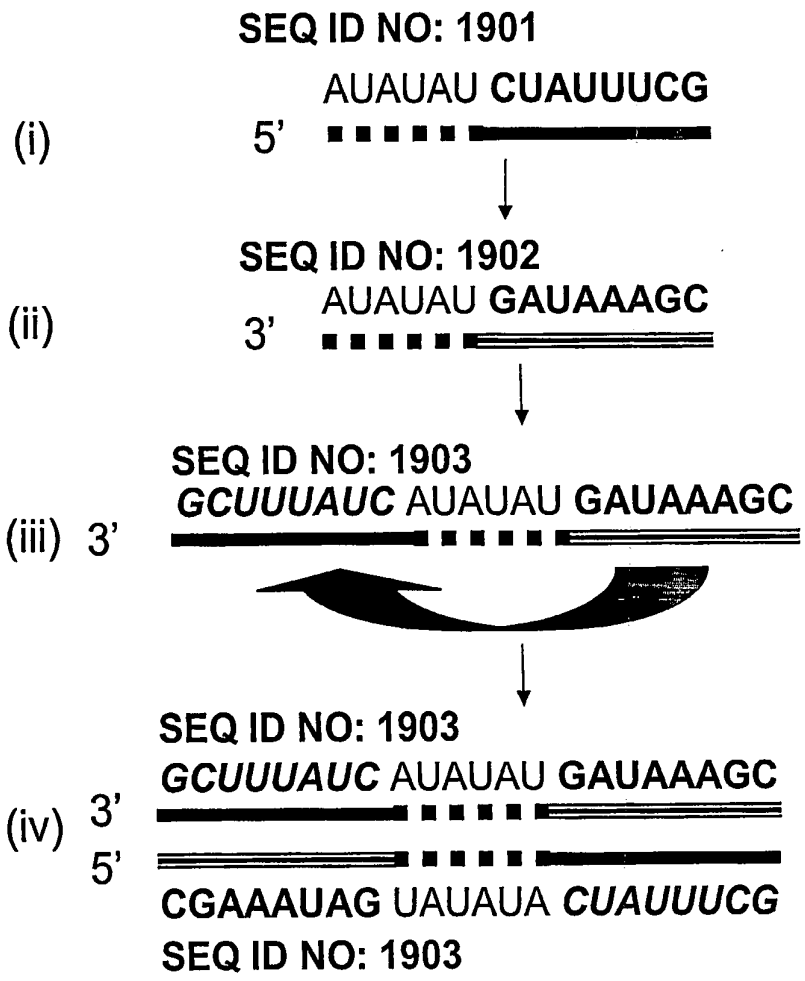


Figure 14B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

Self assembly of self complementary strands to form duplex construct (blunt ends)

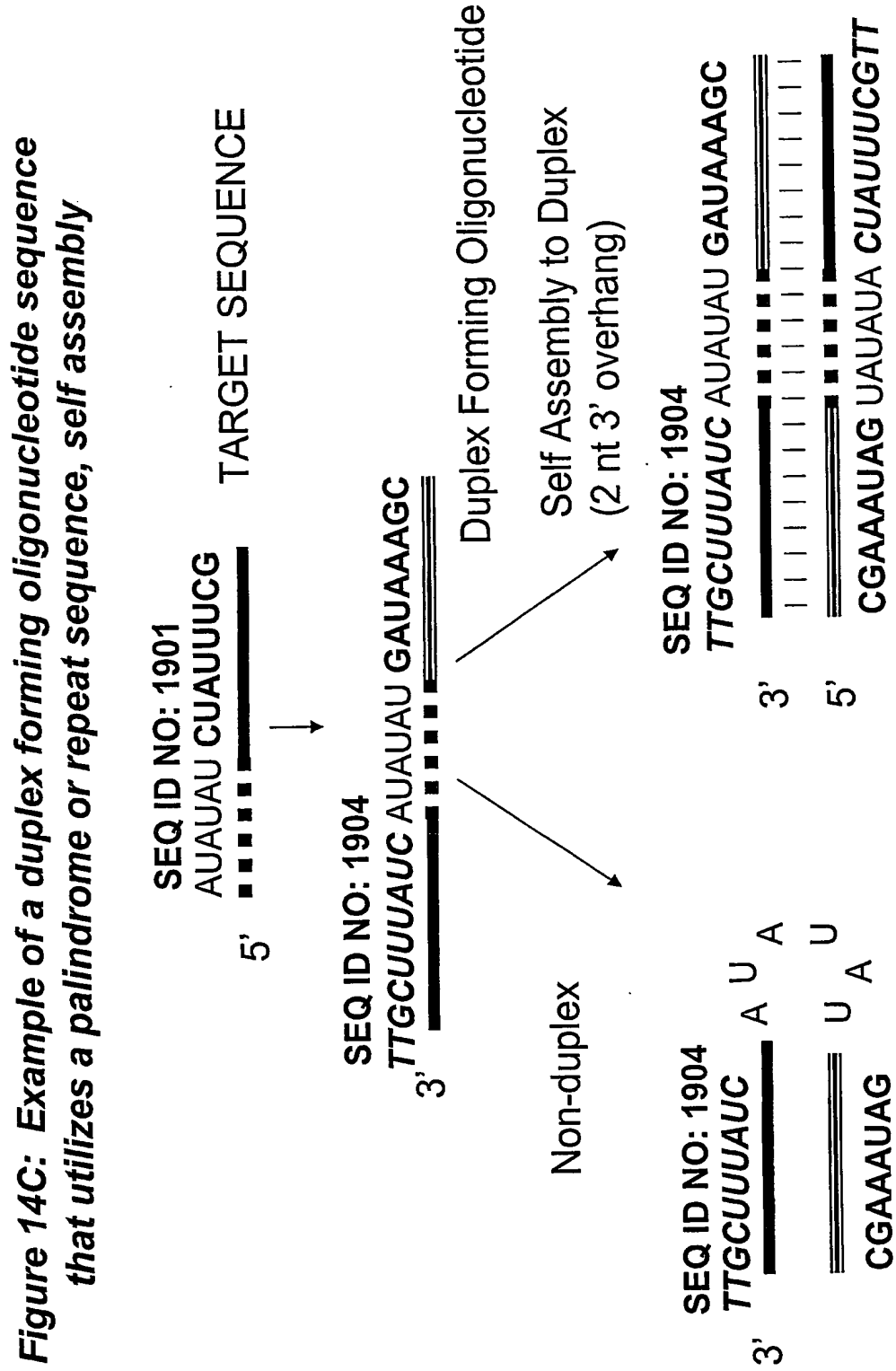


Figure 14C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

Figure 14D: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression

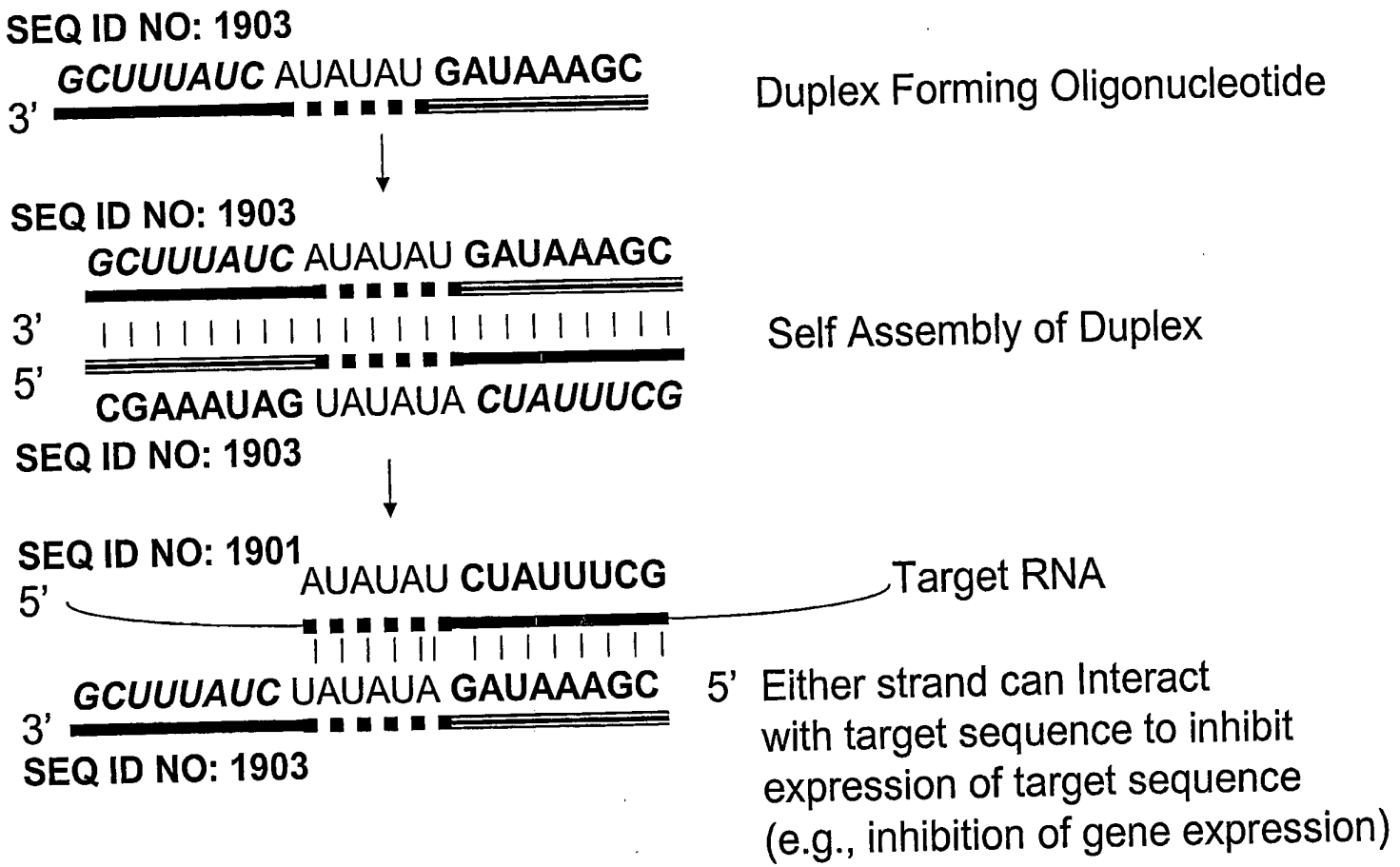


Figure 15: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences

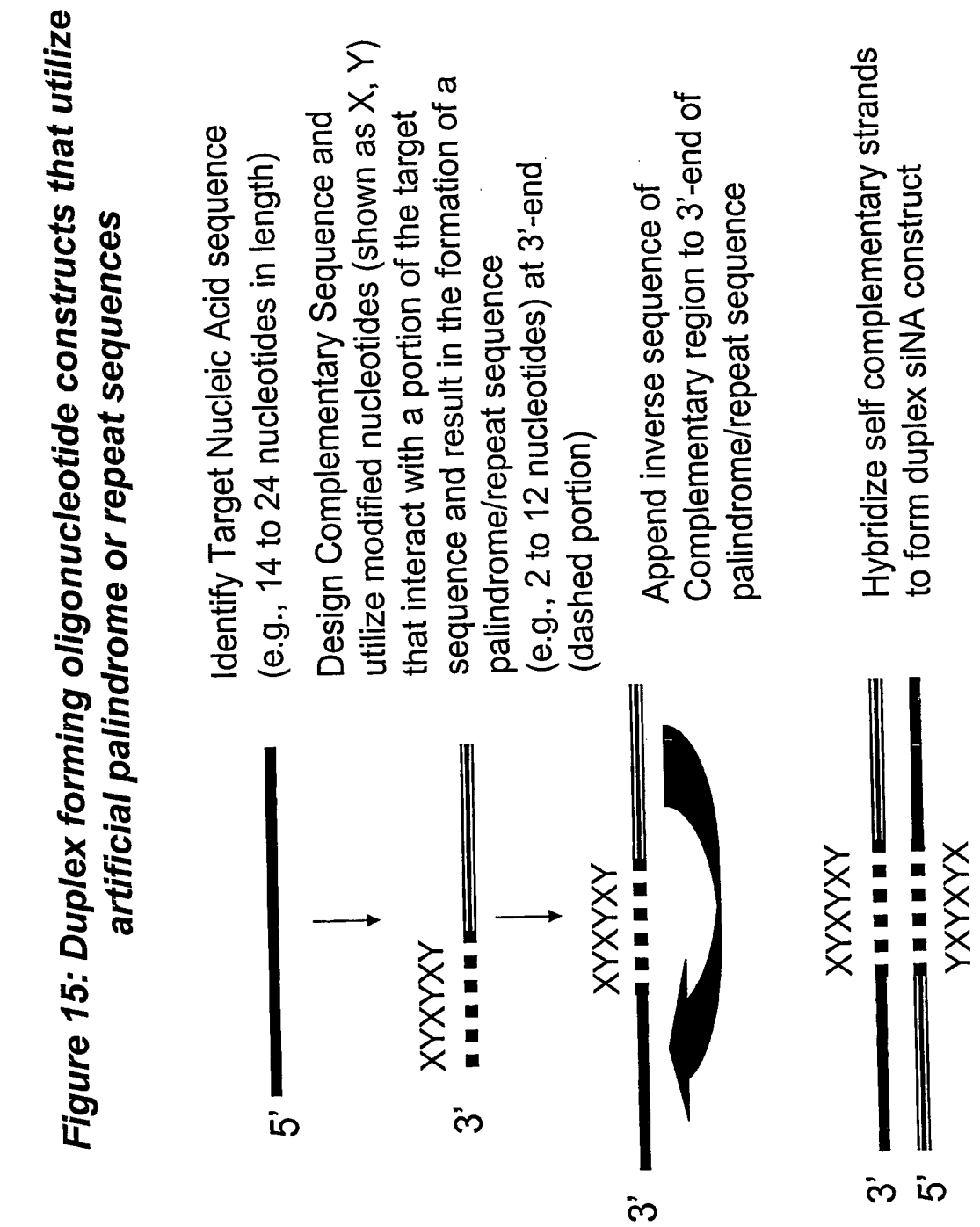


Figure 16: Examples of double stranded multifunctional siNA constructs with distinct complementary regions

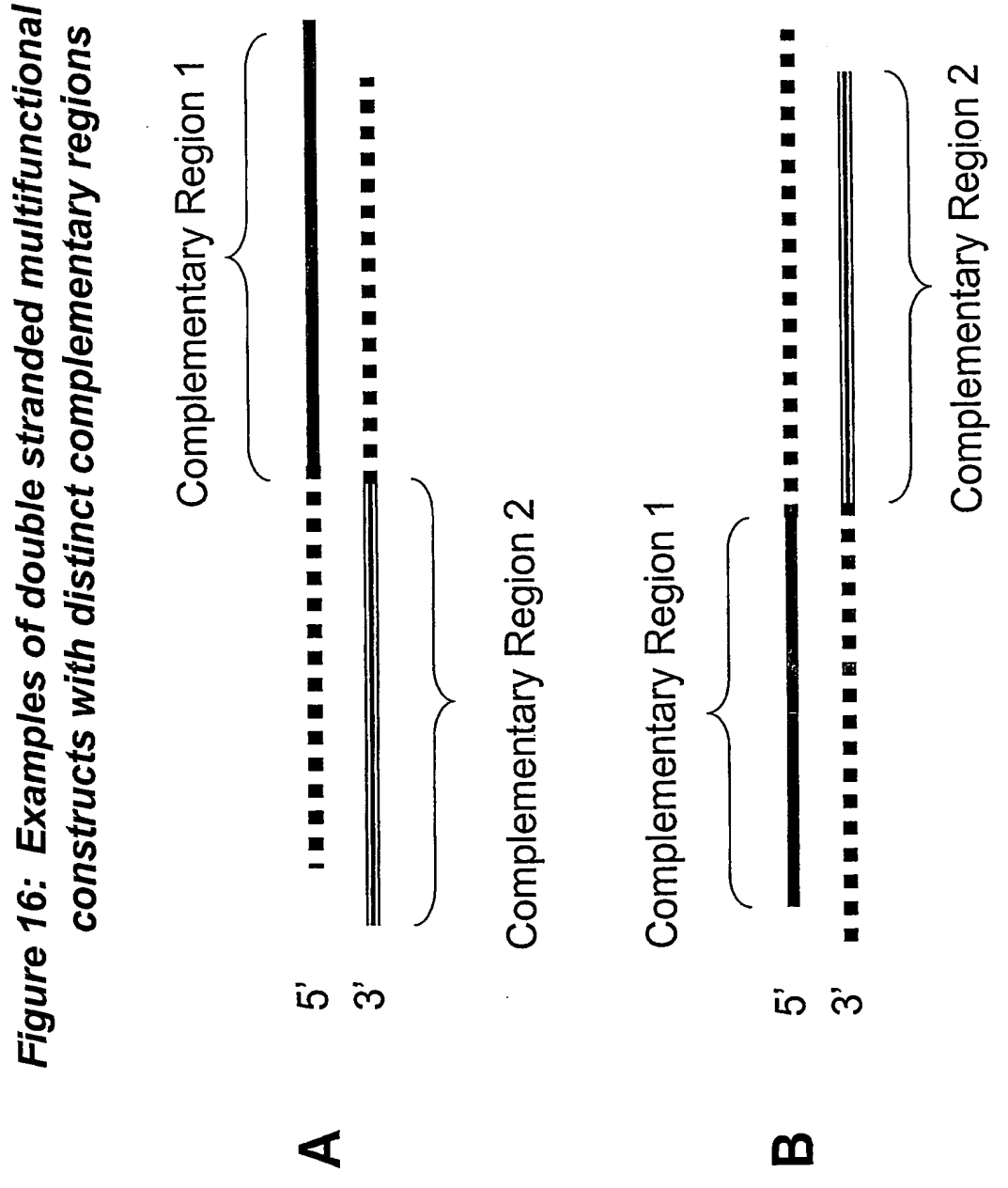


Figure 17: Examples of hairpin multifunctional siNA constructs with distinct complementary regions

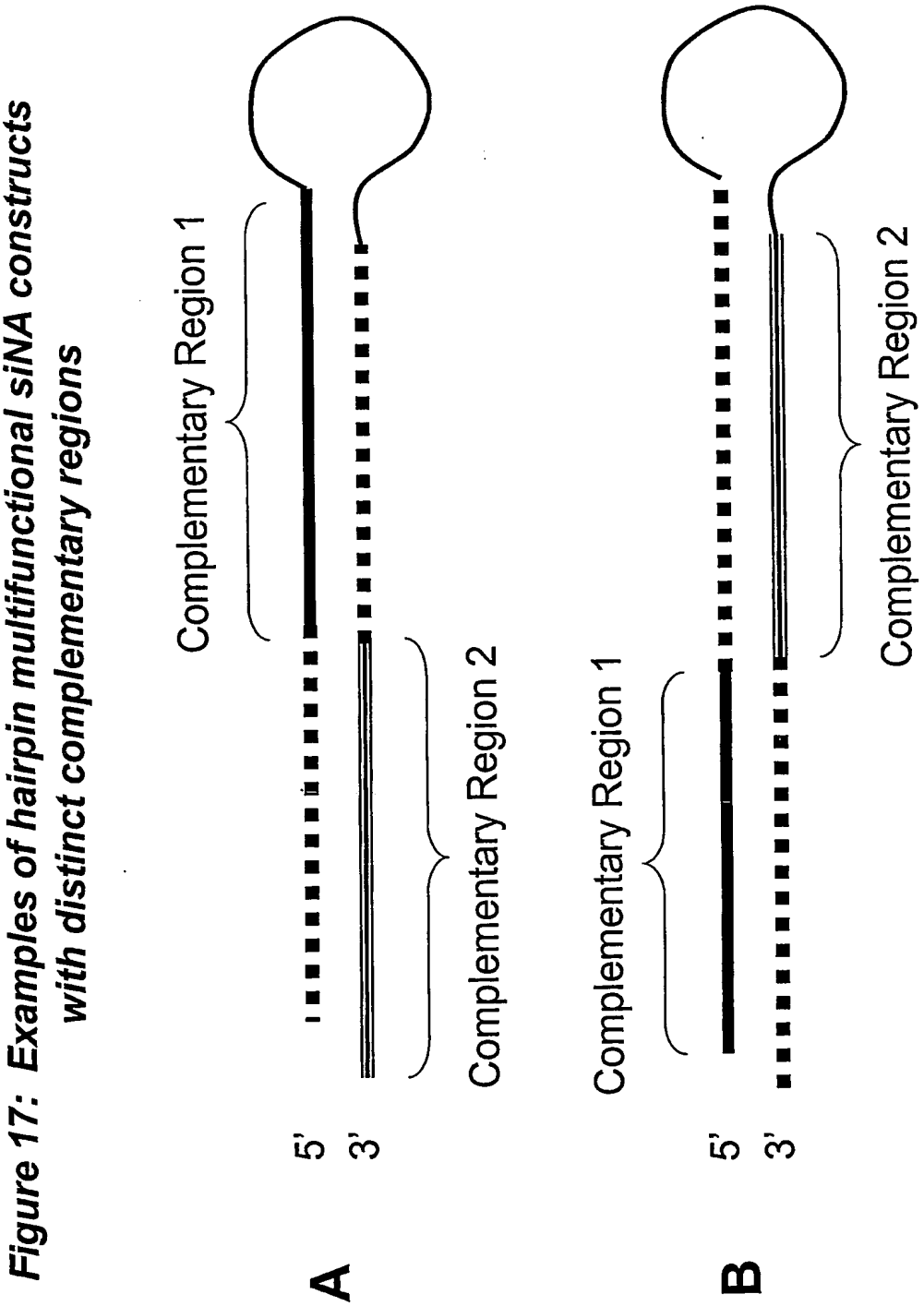
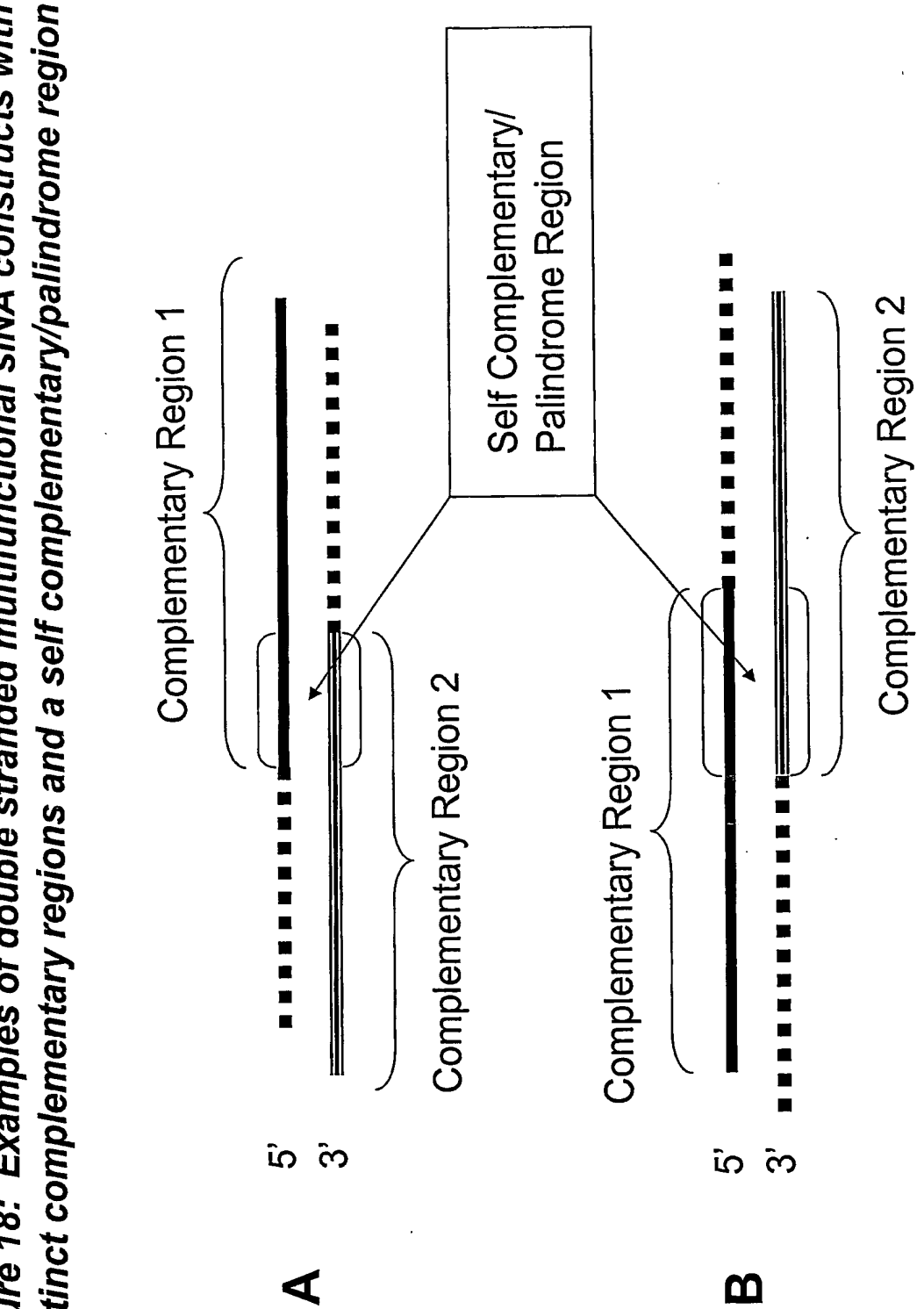


Figure 18: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region



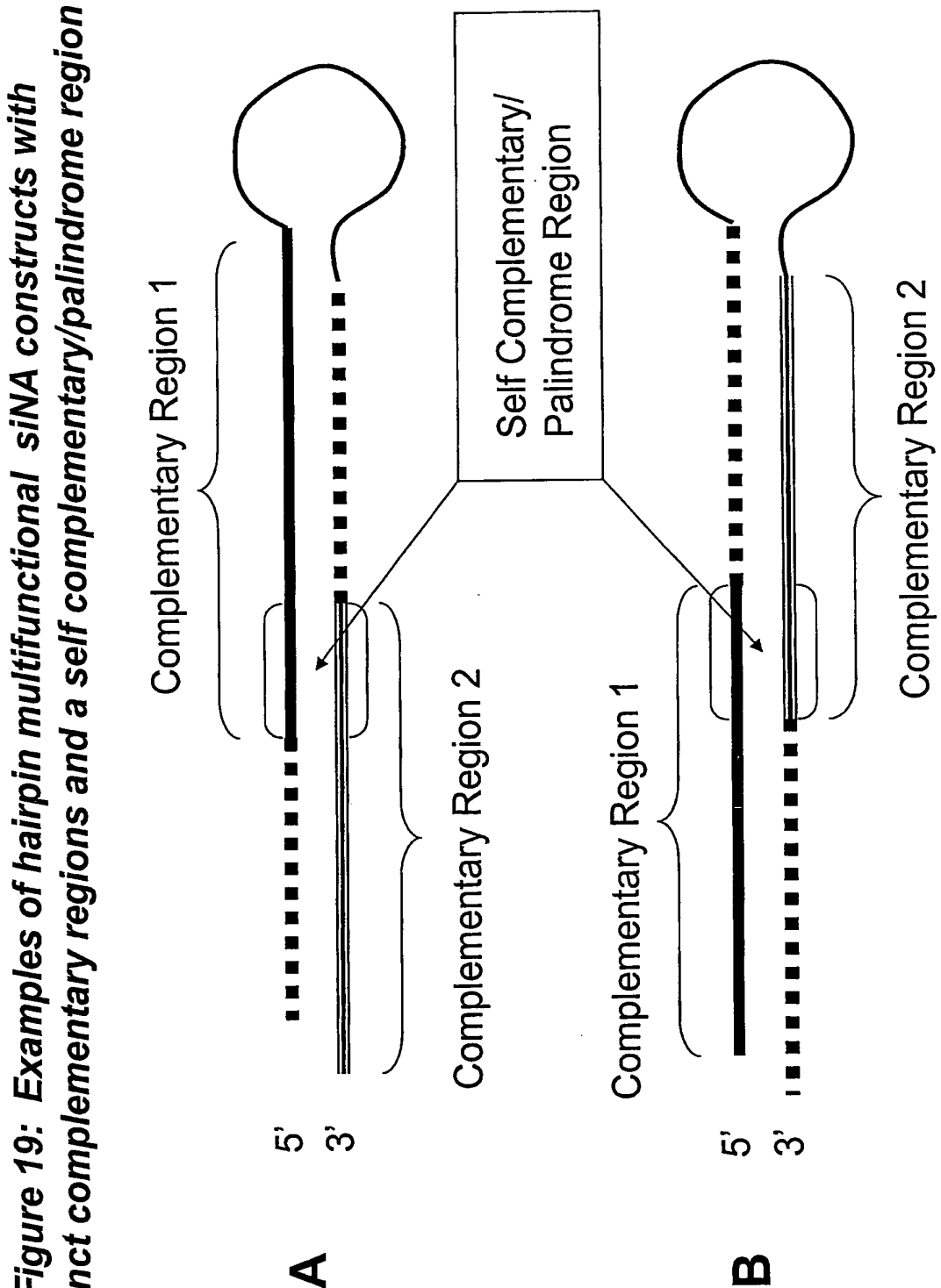


Figure 19: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

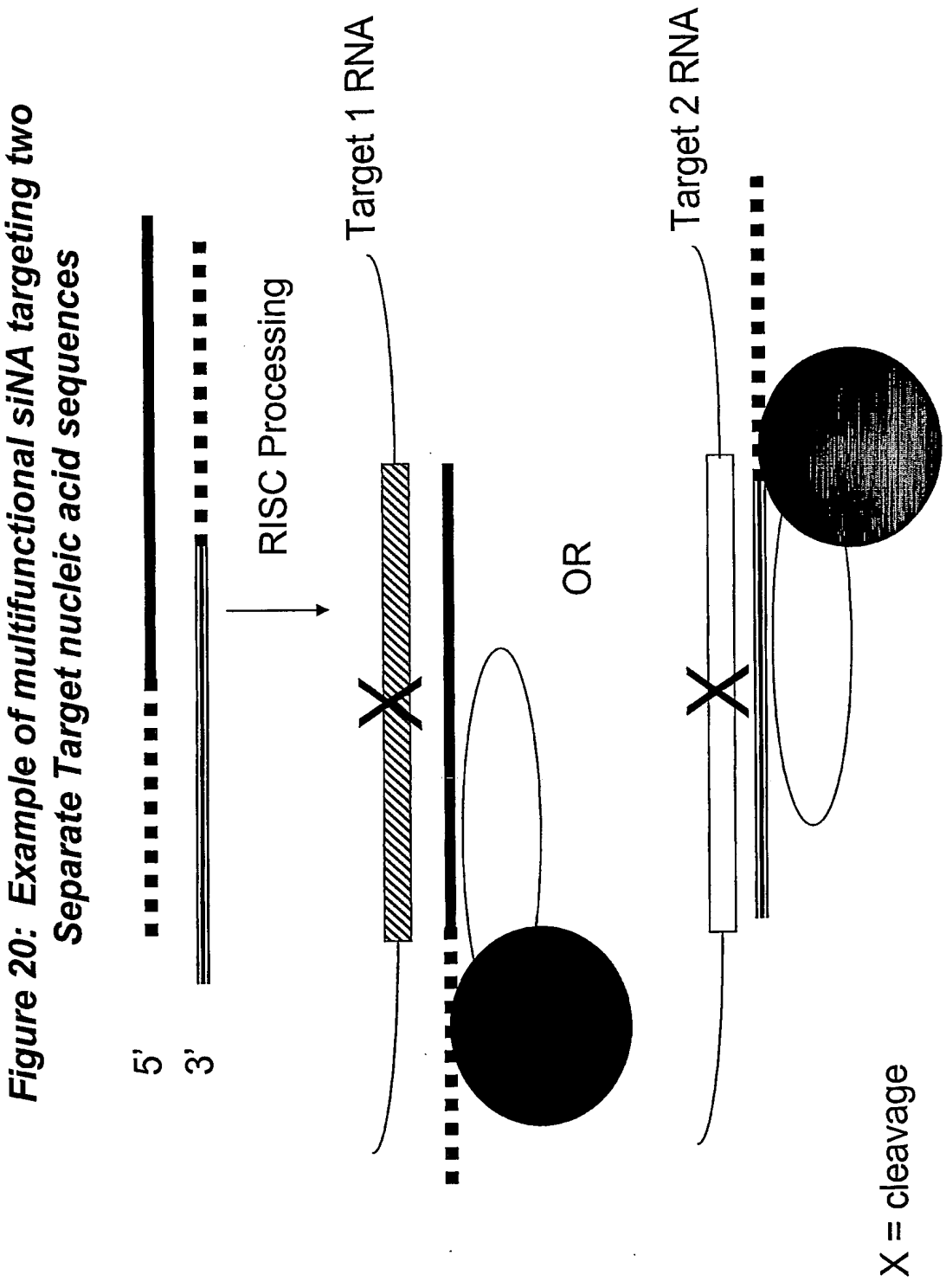
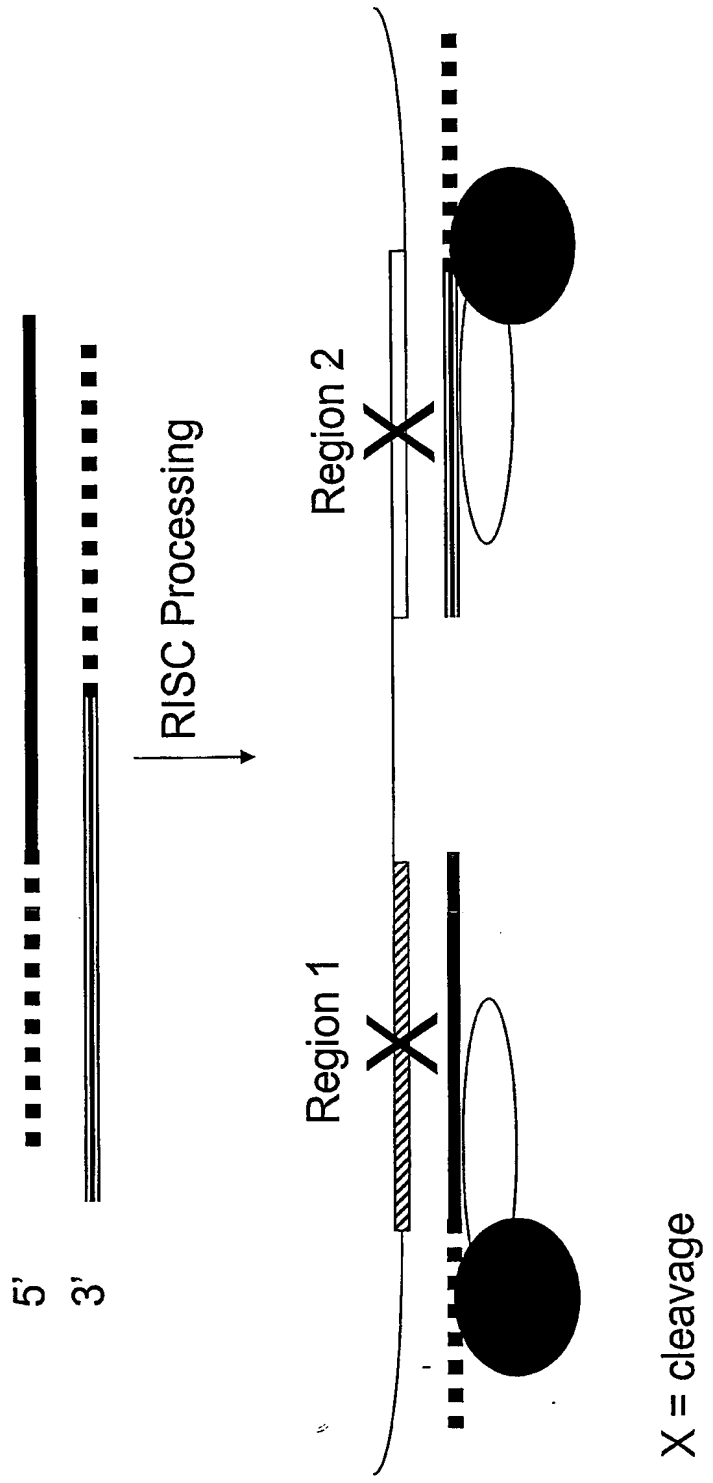


Figure 20: Example of multifunctional siNA targeting two Separate Target nucleic acid sequences

Figure 21: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence



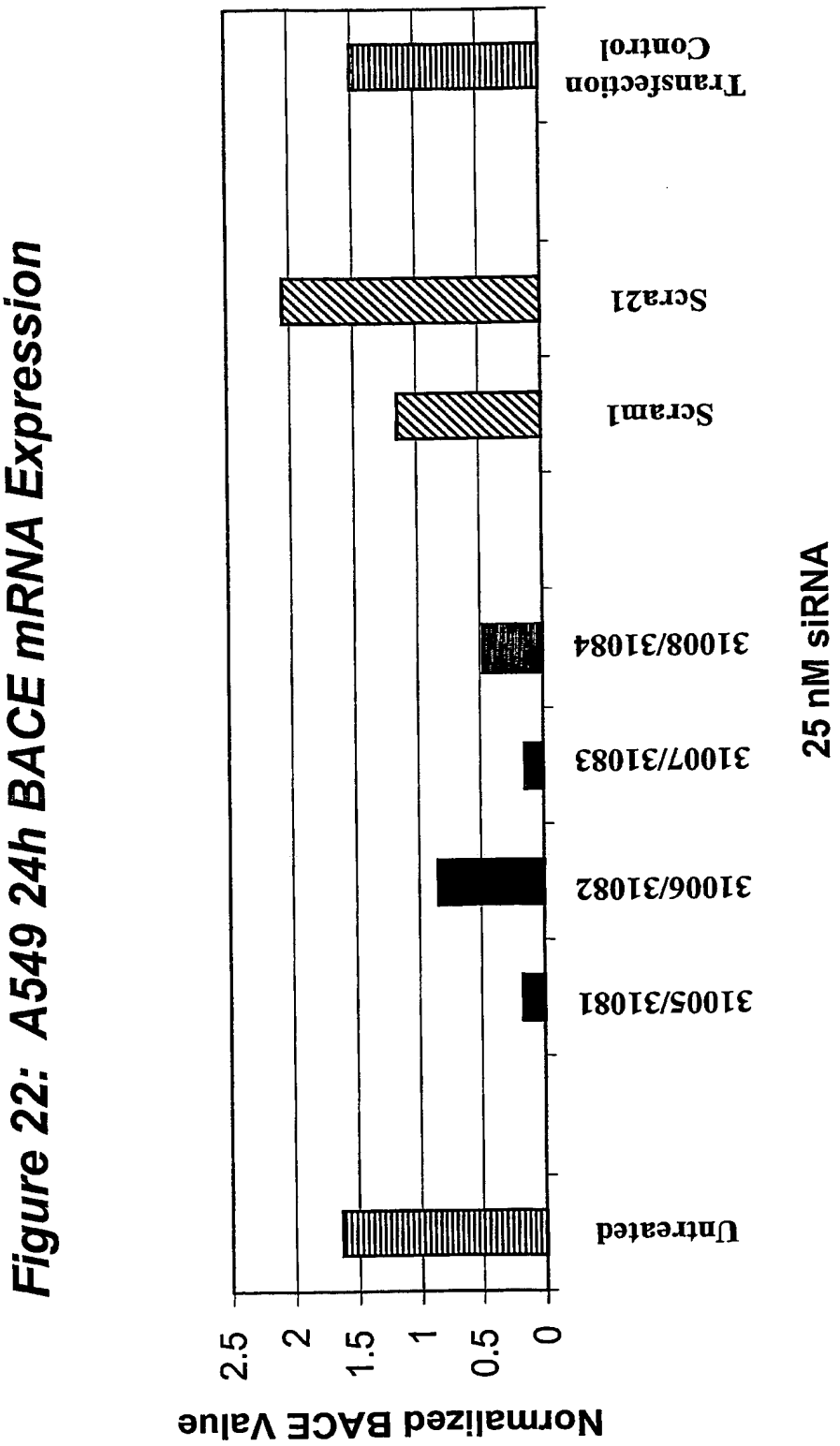


Figure 22: A549 24h BACE mRNA Expression

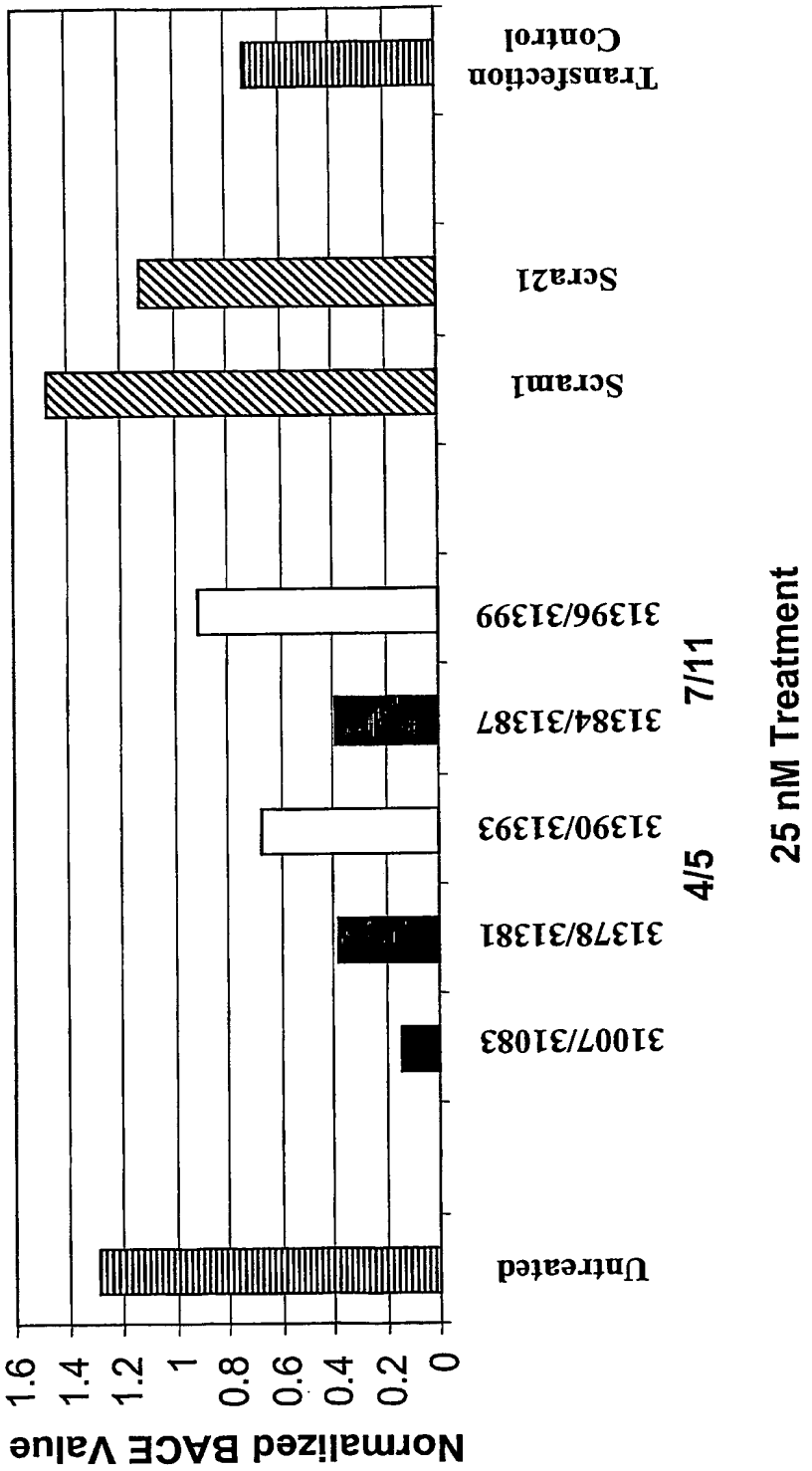


Figure 23: A549 24h BACE mRNA Expression using modified siNA

FIGURE 24

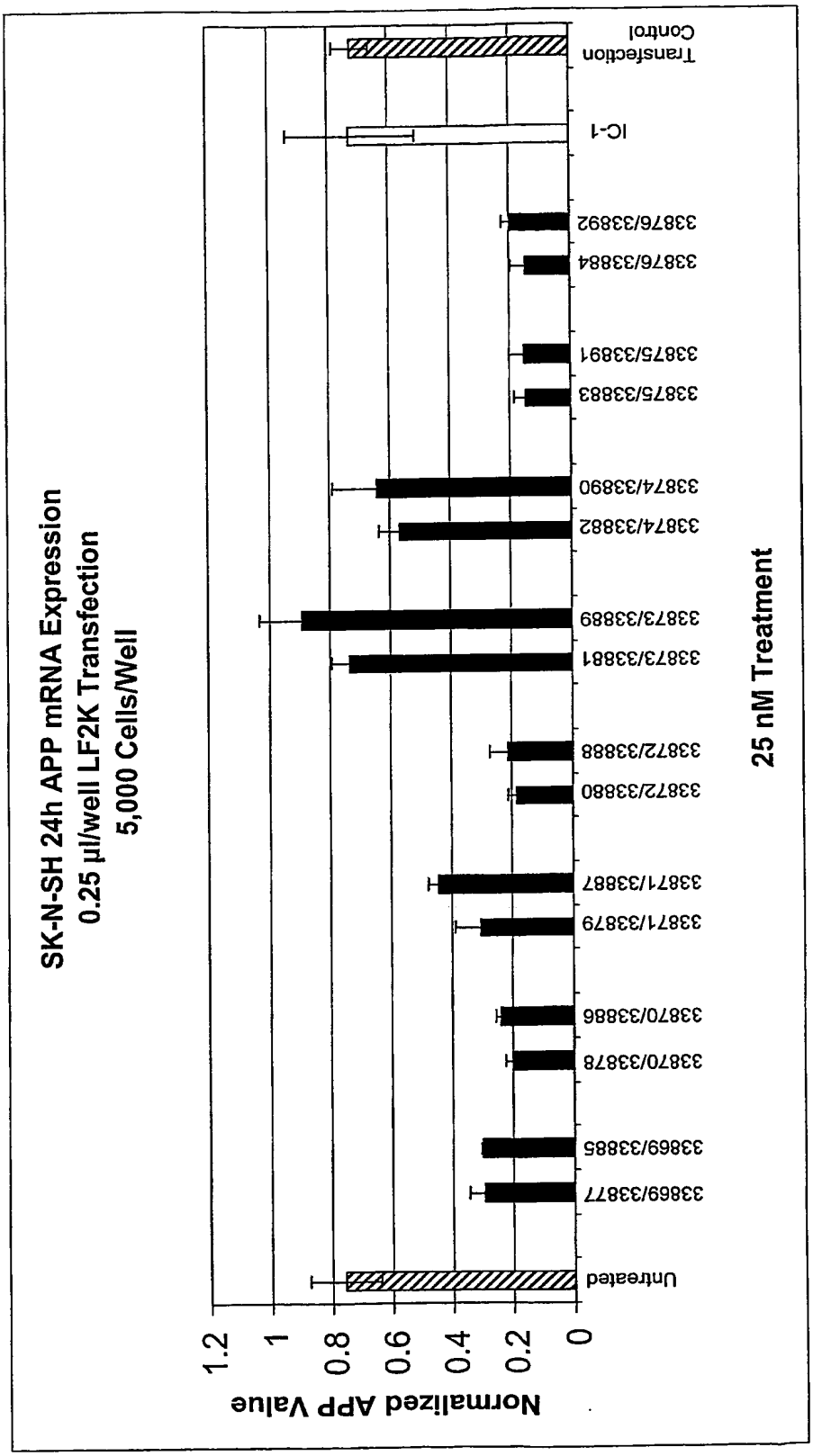

SK-N-SH 24h APP mRNA Expression
0.25 µl/well LF2K Transfection
5,000 Cells/Well
25 nM Treatment
Normalized APP Value
1.2
1
0.8
0.6
0.4
0.2
0
Untreated
33869/33877
33869/33885
33870/33878
33870/33886
33871/33879
33871/33887
33872/33880
33872/33888
33873/33881
33873/33889
33874/33882
33874/33890
33875/33883
33875/33891
33876/33884
33876/33892
IC-1
Transfection Control

FIGURE 25

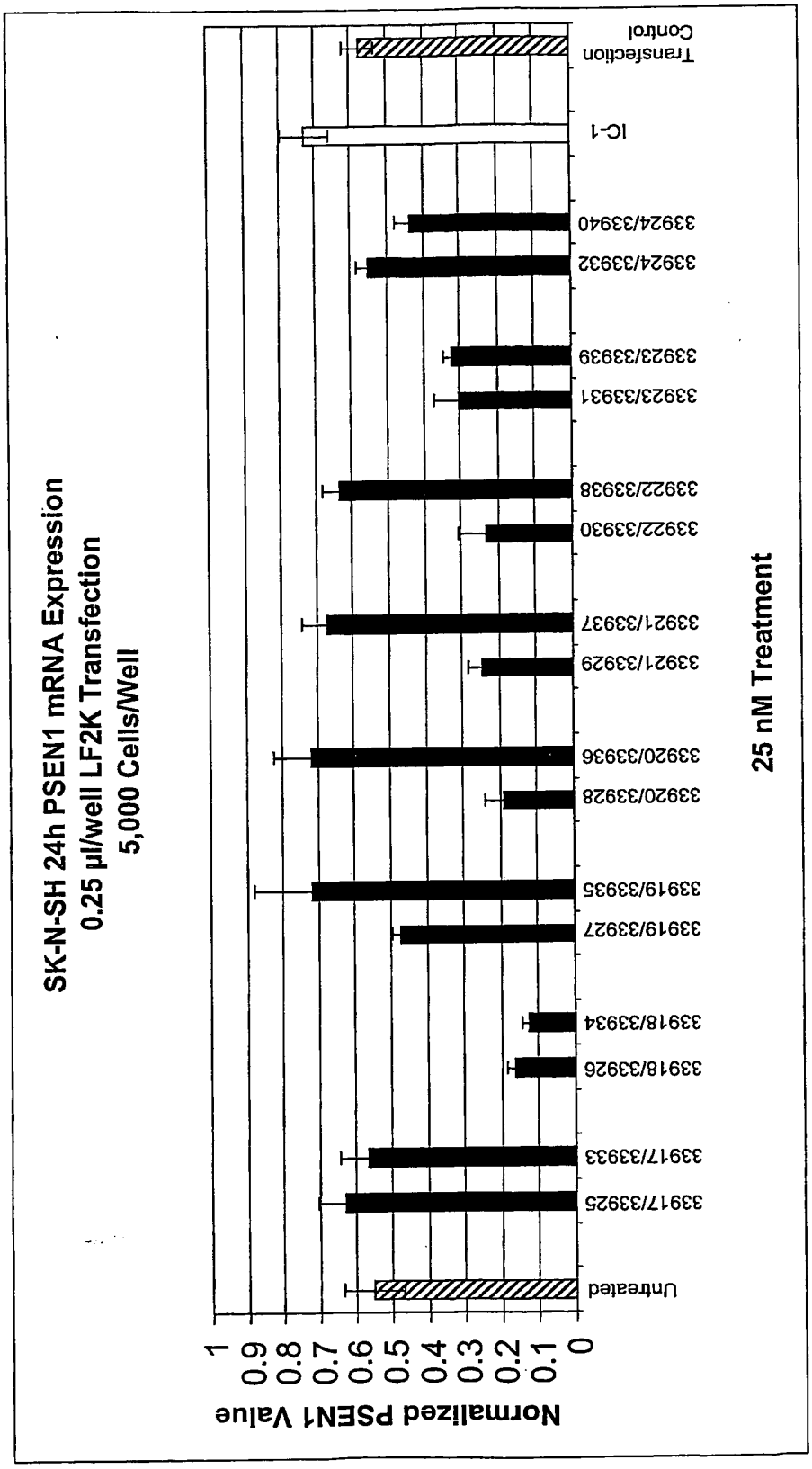
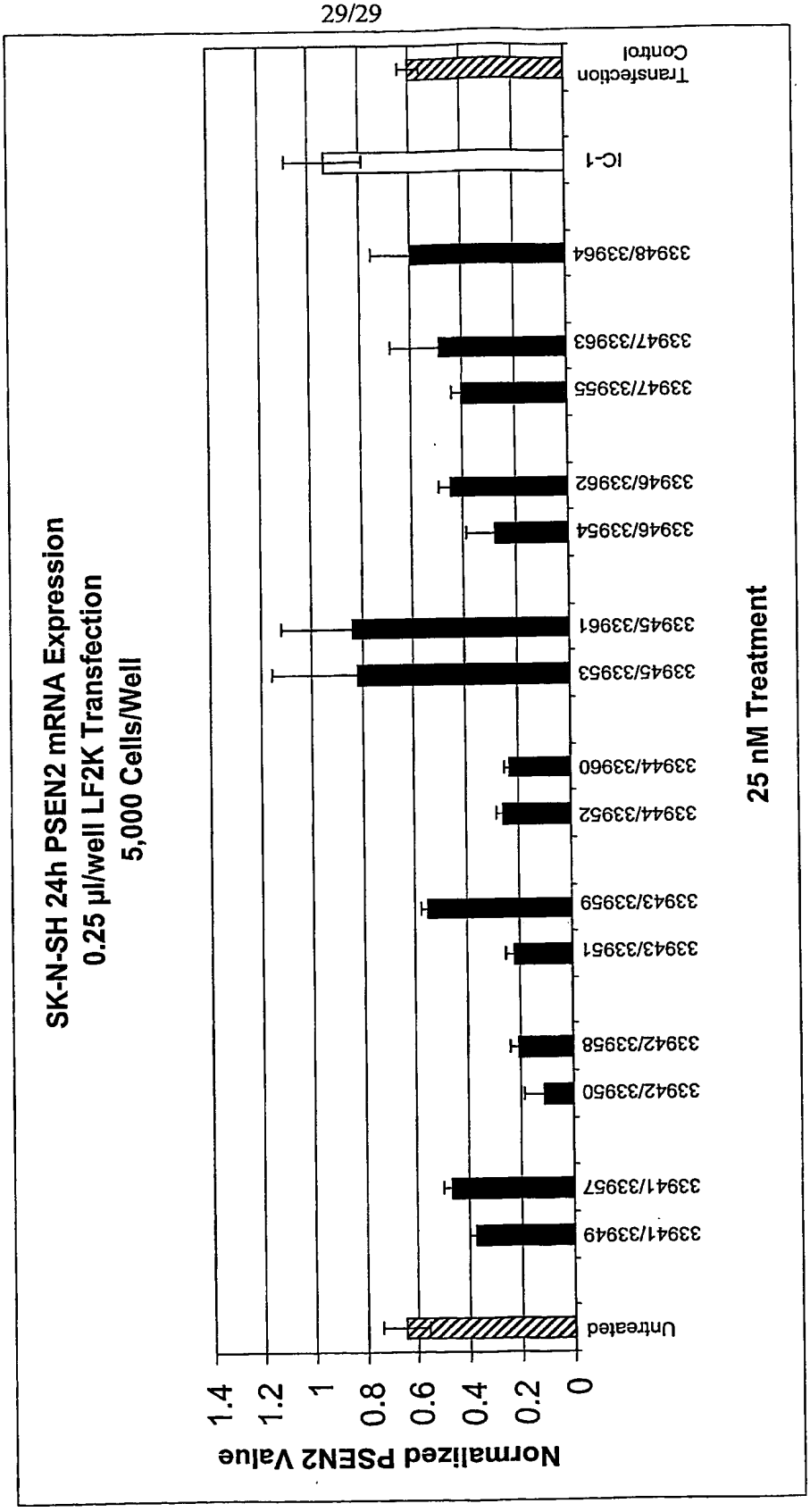

SK-N-SH 24h PSEN1 mRNA Expression
0.25 µl/well LF2K Transfection
5,000 Cells/Well
25 nM Treatment
Normalized PSEN1 Value
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Untreated
33917/33925
33917/33933
33918/33926
33918/33934
33919/33927
33919/33935
33920/33928
33920/33936
33921/33929
33921/33937
33922/33930
33922/33938
33923/33931
33923/33939
33924/33932
33924/33940
IC-1
Transfection Control

FIGURE 26


SK-N-SH 24h PSEN2 mRNA Expression
0.25 µl/well LF2K Transfection
5,000 Cells/Well
25 nM Treatment
Normalized PSEN2 Value
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Untreated
33941/33949
33941/33957
33942/33950
33942/33958
33943/33951
33943/33959
33944/33952
33944/33960
33945/33953
33945/33961
33946/33954
33946/33962
33947/33955
33947/33963
33948/33964
IC-1
Transfection Control